INTERNATIONAL FOOD INFORMATION SERVICE

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FAB 17

MICROBIAL TOXINS IN FOODS

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Under the direction of:-

Commonwealth Agricultural Bureaux, Farnham Royal, Slough; Gesellschaft fur Information und Dokumentation, Frankfurt am Main; Institute of Food Technologists, Chicago; Centrum voor Landbouwpublikaties en Landbouwdocumentatie (Pudoc), Wageningen.

INTRODUCTION

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Coverage of the subject has been restricted to that of Food Science and Technolggy Abstracts, which covers over 1200 of the important food journals, patents from 20 countries and books published world-wide. Every effort is made to include all significant references, but editorial discretion is used on the many articles of borderline interest. If the reader particularly needs an exhaustive search of the subject, we will be pleased to provide any other references that we have available. We would, in any case, encourage readers to write or telephone us with any comments or queries that they may have.

H. BROOKES

EDITOR

1

Thermal inactivation of *Clostridium botulinum* toxin types F and G in buffer and in beef and mushroom patties.

Bradshaw, J. G.; Peeler, J. T.; Twedt, R. M.

Journal of Food Science 46 (3) 688–690, 696 (1981) [24 ref. En] [Div. of Microbiol., FDA, Cincinnati, Ohio 45226, USA]

The time-temp. relationships for the inactivation of crude toxins prepared from *C. botulinum* type F strain Wall 8 and type G strain G89 were determined in 3 different heating menstrua. Toxins diluted to 7700–32 000 mouse LD_{50} units/0.5 ml in beef and mushroom patties (pH 6.05), in 0.1M phosphate buffer (pH 6.05), and in 0.1M acetate buffer (pH 5.0) were heated at temp. from 65.6°–76.7°C. In the beef patties, inactivation times ranged from an average of 108.1 min at 68.3°C to 1.6 min at 76.7°C for type F toxin, and from 158.8 min at 68.3°C to 1.8 min at 76.7°C for type G toxin. Less time was required in phosphate buffer. IFT

2

Food poisoning and salmonellosis surveillance in England and Wales: 1979.

Anon.

British Medical Journal 281 (6251) 1360–1361 (1980) [En]

This brief survey gives details of the incidence of bacterial food poisoning and salmonellosis outbreaks and cases in 1979, the number of deaths involved, location of outbreaks (e.g. restaurants, hospitals, schools), types of food implicated, and *Salmonella* serotypes isolated. A table is included which gives a breakdown of outbreaks (numbers of general outbreaks, family outbreaks and sporadic cases) according to location and causal organism. Numbers of reported cases in 1979 were: salmonellosis 9912 (the highest figure on record); *Clostridium perfringens* food poisoning 1607 (54% more than in 1978); *Staphylococcus aureus* food poisoning 328 (fairly stable figure); *Bacillus cereus* food poisoning 22 (vs. 143 in 1978). The number of general and family outbreaks were, resp. 209 and 325 vs. 203 and 365 in 1978. The number of sporadic cases (mainly salmonellosis) was 7413 vs. 6835 in 1978. Of the 138 general outbreaks of salmonellosis reported, restaurants/hotels, receptions and hospitals accounted for 38, 41 and 24, resp. The types of food implicated were recorded in only some of the outbreaks; *Cl. perfringens* and *Staph. aureus* food poisoning was usually associated with meat other than poultry, while poultry was implicated in many salmonellosis outbreaks and fried rice was the vehicle of infection for *B. cereus* food poisoning. Several incidents involving canned foods were reported, particularly canned corned beef from Argentina, Malta and Brazil contaminated with *Staph. aureus*. Mention is made of a milk-borne outbreak of campylobacter enteritis which affected 2000 people; a failure in the pasteurization plant was thought to be responsible. JA

3

[Toxicology of foods.] Toxikologie der Nahrungsmittel. [Book]

Lindner, E.

Ed. 2, x + 200pp. ISBN 3-13-490802-6 (1979) [De] Stuttgart, Federal Republic of Germany; Georg Thieme Verlag [Univ., Giessen, Federal Republic of Germany]

This revised and expanded edition of this book includes the following chapters: Toxic substances naturally occurring in foods of plant origin (pp. 1–83); Toxic substances naturally occurring in foods of animal origin (pp. 84–90). Mycotoxins (pp. 91–110); Bacterial toxins in foods (pp. 111–117); Toxic substances formed as a result of processing or storage conditions (pp. 117–125); Additives (pp. 126–149); and Food allergy (pp. 150–154). A 35pp. bibliography and an 11pp. subject index are given. AJDW

4

[Coagulase-positive staphylococci.)

Gudkov, A. V.; Slipchenko, S. N.; Goshev, G. N.; Butkus, K. D.

Molochnaya Promyshlennost' No. 10, 19–23 (1980) [8 ref. Ru] [Vses. Nauchno-issled. Inst. Maslodel'noi & Syrodel'noi Promyshlennosti, Uglich, USSR]

This review-type article deals with general aspects of coagulase-positive staphylococci (CS) in milk and milk products. The information given includes data on CS contamination of milk deliveries in 6 dairy factories in different parts of the USSR during 1974–1977, overall mean values for 1850 samples being 824 (44.5%) positive with 274 (14.8%) showing contents of > 10 000/ml; and data on CS contents of deliveries from 6 farms at successive stages from the milking installation to tanker at delivery (total counts, 250 000 and 13.9 million/ml resp; CS counts 280 and 40 000/ml resp.). Data on total and CS counts resp. of milk on 7 farms in Yaroslavl district were as follows: milk stored on the farm for 20 h at 8–10°C before transport, 4.3 million and 460/ml; milk stored for 2–20 h at 12–19°C, 27.2 million and 3500/ml; and milk stored 3–5 h without cooling, 5.6 million and 86 500/ml. SKK

5

[Some aspects of the pathogenicity of enterococci causing food poisoning.]

Sedova, N. N.; Nefed'eva, N. P.; Skirko, B. K.

Voprosy Pitaniya No. 3, 59–61 (1981) [10 ref. Ru, en] [Inst. Pitaniya AMN SSSR, Moscow, USSR]

In connection with potential causes of food poisoning, the enteropathogenicity of enterococci having proteolytic characteristics was studied in experiments on isolated loops of the small intestine of the rat and rabbit. All strains tested, viz. *Streptococcus faecalis* var. *liquefaciens* (4 strains) and *Str. faecalis* var. *zymogenes* (1 strain), possessed enteropathogenic properties. The degree of pathogenicity varied between strains. RAW

6

[Distribution and physiological characteristics of *Bacillus cereus* in rice and rice products.]
Lee, M. S.; Chang, D. S.
Bulletin of the Korean Fisheries Society 13 (4) 163–171 (1980) [32 ref. Ko, en] [Dep. Food Sci. & Tech., Nat. Fisheries Univ. of Busan, Busan, S. Korea]

Out of 101 samples of rice food products collected at the market in Busan, 40 were contaminated with *B. cereus*. 38% of the strains isolated from the 40 samples were positive for haemolysis and negative for starch hydrolysis. The growth rate and generation time of *B. cereus* isolated from cooked rice were 0.34 h^{-1} and 2.02 h at 20°C, 0.73 h^{-1} and 0.95 h at 30°C, and 0.49 h^{-1} and 1.44 h at 40°C, resp. The heat resistance values of the spores of *B. cereus* suspended in phosphate buffer solution were D_{90} = 29.0 min, D_{95} = 8.7 min and D_{101} = 2.3 min (z = 10.5).
SFoST

7

[Biochemical and toxic properties of *Staphylococcus epidermidis* strains isolated from cows' milk.]
Nowakowski, W.
Medycyna Weterynaryjna 36 (1) 39–42 (1980) [35 ref. Pl, ru, en] [Zaklad Higieny Weterynaryjnej, Katowice, Poland]

Milk samples from 1230 cows which gave a positive test in the field cell reaction (TOK) were examined. 176 of 530 strains of Gram-positive cocci isolated were identified as *Staphylococcus aureus, Micrococcus* spp. and microorganisms not belonging to the Micrococcaceae. The remaining 354 strains were identified as *S. epidermidis*; 272 of these strains showed marked ability to ferment mannitol and lactose anaerobically and are provisionally described as *S. epidermidis* subsp. *mastitis*, their properties being similar to those of *S. saprophyticus*. SKK

8

[Determination of the count of coagulase-positive staphalococci in fishery products within 24 hours. II.]
Suntjens, M. A. M.; Spreekens, K. J. A. van
Voedingsmiddelentechnologie 14 (14) 11–14 (1981) [7 ref. Nl, en] [Afdeling Inst. voor Visserijprodukten TNO, Ijmuiden, Netherlands]

Use of the pour plate technique with Toepoel & Van Spreekens' double layer plate (bovine fibrinogen agar covered with porcine plasma agar) [see FSTA (1981) 13 5R241] was evaluated. 33 samples of fishery products (mainly imported frozen shrimps, steamed mussels and breaded plaice fillets) were evaluated by this technique and on the conventional Baird Parker medium. Tables of results are given. With the double layer plate, pour plating gave appreciably higher counts than streak plating. Pour-plating on the double layer plates gave counts which agreed well with those on Baird-Parker medium. All typical colonies on the double-layer plates were confirmed to be coagulase-positive, and all atypical colonies were coagulase-negative. On Baird-Parker medium, 5% of typical colonies were coagulase-negative and 23% of atypical colonies were coagulase-positive. AJDW

9

Production of thermonuclease deficient mutants from an enterotoxigenic thermonuclease producing strain of *Staphylococcus aureus*.
Batish, V. K.; Chander, H.; Ghodekar, D. R.; Ranganathan, B.
Journal of Food Science 46 (4) 1252–1254 (1981) [23 ref. En] [Dairy Bacteriology Div., Nat. Dairy Res. Inst., Karnal 132 001, India]

A toxigenic strain of *S. aureus* (S_6) known to produce both thermonuclease and enterotoxin was exposed to UV radiation. Thermonuclease deficient mutants were recovered on methylgreen-DNA-agar medium. All of 20 thermonuclease deficient mutants lost the capacity to produce coagulase, enterotoxin, and lecithinase. However, these functions were regained upon reversion with visible light (photoreactivation) thus suggesting a coordinated control mechanism for the synthesis and release of the above metabolites. These characteristics in most of the mutants remained stable for 3 months. IFT

10

[The main hazard: salmonellae.] Hauptgefahr: Salmonellen.
Anon.
GV-Praxis mit Tiefkühlpraxis 20 (8) 62–64 (1980) [De]

The food poisoning situation in the Federal Republic of Germany in 1980 is discussed, with reference to tables of data. Of 80 outbreaks, 16 were in restaurants, 35 in canteens, 15 in hospitals and 14 in the home; 66.5% of all patients were from outbreaks in canteens. Salmonellae were responsible for 79.8% of outbreaks, staphylococci for 10.1%, *Clostridium botulinum* for 4.8%, and *Cl. perfringens* for 1.3%. Most outbreaks were attributable to incorrect cooling of foods, or warm-holding of prepared foods. AJDW

11

[Food poisoning in the Federal Republic of Germany as seen in the literature.] Lebensmittelvergiftungen in der Bundesrepublik Deutschland im Spiegel der Literatur. [Review]
Sinell, H. J.; Kolb, F.
Fleischwirtschaft 61 (4) 572, 574–577; 606 (1981) [62 ref. De, en] [Inst. für Lebensmittelhygiene, Fleischhygiene & -technologie, Freie Univ., Berlin]

229 outbreaks of food poisoning reported in the Federal Republic of Germany between 1968 and 1978 and affecting 12 268 persons are analysed. Tabulated data show that nearly 80% of the reports are concerned with salmonella infections (182 outbreaks, affecting 8451 persons). 17.6% of the outbreaks (2165 persons) were due to staphylococci, 7.8% (959 persons) to *Clostridium perfringens*. The main foods involved were meat and meat products (40.3%), poultry (14.3%), dairy products and ice cream (10.5%). Egg products and fish + shellfish accounted for only 5% each. The most frequent cause was inadequate heat treatment. The fact that salmonellosis is the subject of most of the reports is attributed to the Central Salmonellosis Control Programme. Other intoxications may be underrepresented and require more systematic reporting, to allow control measures to be devised. RM

12

Microbiological problems in dairy foods in the 1980s. [Review]
Richter, R.
Journal of Food Protection 44 (6) 471–475, 479 (1981) [67 ref. En] [Dep. of Anim. Sci., Texas A&M Univ., College Station, Texas 77843, USA]

Deterioration of quality in dairy products, the microbiological problems encountered in new dairy food manufacturing processes, and bacterial and chemical toxicity in dairy foods are reviewed. JMa

13

[A case of botulism – special aspect of food examination.] Ein Fall von Botulismus unter dem besonderen Aspekt der Diagnose am Lebensmittel.
Schulze, K.; Funke, R.
Archiv für Lebensmittelhygiene 32 (3) 83–87 (1981) [many ref. De, en] [Veterinäruntersuchungsamt des Landes Schleswig-Holstein, 2350 Neumünster, Federal Republic of Germany]

A case of botulism following consumption of raw cured smoked ham is described. *Clostridium botulinum* serotype B was isolated from the suspect ham. The strain was able to grow and produce toxin at room temp. and at 11°C. Growth was greatly inhibited at 37°C. AS

14

The causes of microbial food poisoning.
Lücke, F.-K.
Fleischerei 32 (6) VII–VIII (1981) [En] [Bundesanstalt für Fleischforschung, E.-C.-Baumann-Strasse 20, 8650 Kulmbach, Federal Republic of Germany]

Organisms causing food poisoning (salmonellae, *Clostridium perfringens, Staphylococcus aureus, Clostridium botulinum*) are discussed, with reference to: descriptions of the organisms; type of poisoning caused; incidence of contamination of food; causes and sources of contamination; and products likely to be responsible for food poisoning. AJDW

15

[Correlation between output of enterotoxins and thermostable nuclease by strains of *Staphylococcus aureus* isolated from various environmental objects.]
Bugrova, V. I.
Voprosy Pitaniya No. 3, 56–58 (1981) [4 ref. Ru, en] [Inst. Pitaniya AMN SSSR, Moscow, USSR]

Enterotoxins and thermostable DNase occurred concurrently in 45.7% of *Staphylococcus aureus* strains of food samples; DNase occurred without enterotoxins in 45.6% of these samples. enterotoxins occurred without DNase in 5.7%, and neither was detected in 3.0% of samples. Accuracy of the results was confirmed in tests on concn. food samples (cheese, tvorog, preserved 'sprats in oil') responsible for staphylococcal food poisoning. Bacteriological tests showed that presence of heat-stable DNase alone was not always indicative of presence of enterotoxins. RAW

16

Endogenous radiolabeling of enterotoxin from *Clostridium perfringens* type A on a defined medium.
Granum, P. E.; Skjelkvale, R.
Applied and Environmental Microbiology 42 (4) 596–598 (1981) [17 ref. En] [Norwegian Food Res. Inst., N-1432 As-NLH, Norway]

17

Vacuum-packed cooked potatoes: toxin production by *Clostridium botulinum* and shelf life.
Notermans, S.; Dufrenne, J.; Keijbets, M. J. H.
Journal of Food Protection 44 (8) 572–575, 579 (1981) [16 ref. En] [Lab. for Zoonoses & Food Microbiol., Nat. Inst. of Public Health, PO Box 1, 3720 BA Bilthoven, Netherlands]

Spores of both proteolytic and non-proteolytic strains of *Cl. botulinum* (types A, B, C and E) were subjected to various studies including: detn. of thermal inactivation in phosphate buffer and in vacuum-packaged cooked potatoes; detn. of toxin production in potato medium (mixture of potato pieces and NaCl solution); detn. of survival and toxin production in vacuum-packaged potatoes subsequently cooked at 95°C for 40 min and stored in the dark at 4–20°C. Further studies examined the shelf-life of and aerobic mesophilic bacterial count in uninoculated vacuum-packaged cooked potatoes stored at 2–20°C for ≤44 days. Results, which are detailed in graphs and tables, included the following. Thermal inactivation of spores was slower in potatoes than in phosphate buffer. All proteolytic strains produced toxin in potato medium. Shelf-life was inversely related to storage temp., e.g. at 2–4°C, shelf-life was ≥6 wk, while at 15° and 20°C, some pouches were swollen after a few days. Toxin production in vacuum-packaged cooked potatoes varied depending on the *Cl. botulinum* strain present and the storage temp.; at 10–20°C, toxin production occurred before the product was spoiled. It is therefore concluded that the potential public health risk with regard to botulism can be eliminated only if the vacuum-packaged cooked potatoes are stored at ≤4°C JA

18

Evaluation of the botulism hazard from imitation cheeses.
Kautter, D. A.; Lynt, R. K.; Lilly, T., Jr.; Solomon, H. M.
Journal of Food Science 46 (3) 749–750, 764 (1981) [6 ref. En] [Div. of Microbiol., FDA, Dep. of Health & Human Services, Washington, DC 20204, USA]

11 imitation cheeses (in which milk components are partially or completely replaced by nonmilk components] were studied for their ability to support growth and toxin production of *Clostridium botulinum* types A and B at 26°C. The a_w of the cheeses ranged from 0.942 to 0.973 and the pH from 5.52 to 6.14. Spores of 5 type A and 5 type B strains were inoculated into approx. 50 samples of each cheese. In 289 days only one type, an imitation mozzarella, became toxic. In 2 separate tests, 27 of 77 samples of imitation mozzarella contained toxin. The earliest toxic sample was noted in 26 days; however, the product was organoleptically unacceptable. Imitation cheese thus appears to pose no hazard from botulism, even under conditions of abuse. IFT

19

Inhibition of growth of *Staphylococcus aureus* and enterotoxin-A production in Cheddar cheese produced with induced starter failure.

Ibrahim, G. F.; Radford, D. R.; Baldock, A. K.; Ireland, L. B.

Journal of Food Protection 44 (3) 189–193, 200 (1981) [21 ref. En] [Dep. of Agric. Dairy Res. Cent., PO Box 217, Richmond, NSW 2753, Australia]

12 batches of Cheddar cheese were produced from HTST pasteurized milk containing 100 colony-forming units (c.f.u.) *S. aureus*/ml, using 1.5% starter inoculum and specific bacteriophage to prevent starter activity. The cheeses were stored at 4° or 11°C. After pressing, the mean moisture content in unsalted and salted cheeses was 45.76 and 44.09%, resp. and pH was 6.46 and 6.57, resp.; during storage, the pH decreased more ($P < 0.05$) in unsalted than in salted cheeses and was 5.42 and 6.20 resp. after 6 wk at 11°C. At the end of pressing, the *S. aureus* count had reached about 10^8 c.f.u./ml and was higher in unsalted than in salted cheeses. During the 1st 2 wk of storage at 11°C, *S. aureus* count decreased in unsalted cheese but increased in salted cheese; at 4°C, counts decreased in unsalted and, to a lesser extent, in salted cheeses. Enterotoxin was detected at the end of pressing, concn. being lower ($P < 0.01$) in unsalted than in salted cheeses; contents increased in salted cheeses during storage at 11°C but remained virtually unchanged in other cheeses. Flavour defects developed at 11°C but only occasionally at 4°C. CDP

20

Effect of modified atmosphere storage on *C. botulinum* toxigenesis and the spoilage microflora of salmon fillets.

Stier, R. F.; Bell, L.; Ito, K. A.; Shafer, B. D.; Brown, L. A.; Seeger, M. L.; Allen, B. H.; Porcuna, M. N.; Lerke, P. A.

Journal of Food Science 46 (6) 1639–1642 (1981) [En] [Tech S Corp., 1950 Sixth Street, Berkeley, California 94710, USA]

Modified atm. (MA) storage in conjunction with refrigeration has been shown to significantly increase the shelf life of fresh fish and other products, but the effects, if any on the outgrowth and toxigenesis of *Clostridium botulinum* are unknown. A commercial system was duplicated in the laboratory and the effects of MA on the outgrowth of *C. botulinum* types A, B, and E were observed in inoculated salmon fillets and sandwiches stored at 4.4° and 22.2°C. Inoculated samples stored in air at the same temp. were used as controls. No toxigenesis was observed in either the air or modified samples stored at 4.4°C, but all inoculated samples held at 22.2°C were toxic within 2–3 days. Spoilage generally preceded toxigenesis. In a concurrent study, the tendency of CO_2 environments to repress the growth of Gram negative bacteria to a greater extent than Gram positive bacteria was also noted. IFT

21

Growth and enterotoxin A production by *Staphylococcus aureus* in precooked bacon in the intermediate moisture range.

Lee, R. Y.; Silverman, G. J.; Munsey, D. T.

Journal of Food Science 46 (6) 1687–1692, 1700 (1981) [En] [Sci. & Advanced Tech. Lab., US Army Natick Res. & Development Lab., Natick, Massachusetts 01760, USA]

Different water activities were obtained in precooked bacon by varying the frying time. Water activity (a_w) correlated best to the moisture, salt and protein content. When stored aerobically at 37°C, *S. aureus* A100 was capable of rapid growth in precooked bacon at $a_w \geq 0.84$, whereas at 20°C $a_w \geq 0.88$ was required. Under anaerobic storage at 37°C, growth was observed at a_w 0.90, and at 20°C slight growth was noted at a_w 0.91. The increase in the min. a_w required for aerobic growth at the lower temp. was reflected in the differences between the isotherms obtained at 37°C and 20°C. The max. populations achieved were higher for samples stored aerobically. Enterotoxin A (19–821 ng/g) was found in all aerobically stored samples where growth occurred. Enterotoxin A (38–109 ng/g) was also found in all anaerobically incubated samples where the population of *S. aureus* increased more than one logarithmic cycle. IFT

22

Prediction of toxin production by *Clostridium botulinum* in pasteurized pork slurry.

Roberts, T. A.; Gibson, A. M.; Robinson, A.

Journal of Food Technology 16 (4) 337–355 (1981) [24 ref. En] [/ gric. Res. Council, Meat Res. Inst., Langford, Bristol BS18 7DY, UK]

A model pork slurry system was used to study factors controlling the growth of *C. botulinum* types A and B. The following factors were studied in combination: NaCl (2.5, 3.5, 4.5% w/v on water); $NaNO_2$ (100, 200, 300 μg/g); (0, 500 μg/g); sodium isoascorbate (0, 1000 μg/g); polyphosphate (Curaphos 700, 0, 0.3% w/v); heat treatment (none, 80°C/7 min, 80°C/7 min + 70°C/1 h); at 2 pH levels and stored at: 15°, 17.5°, 20° or 35°C. Analyses of results yielded a statistical model providing 2 formulae (for 'low' and 'high' pH slurries) which estimate the probability of toxin production in the pork slurry system within the limits defined above. Values for the probability are tabulated for all the combinations of variables listed. AS

23

Extraction and separation of staphylococcal enterotoxin in foods: collaborative study.

Bennett, R. W.; McClure, F.

Journal of the Association of Official Analytical Chemists 63 (6) 1205–1210 (1980) [12 ref. En] [Div. of Microbiol., FDA, Washington, DC 20204, USA]

The method currently used by the FDA for the extraction and chromatographic separation of *Staphylococcus aureus* enterotoxins from foods was evaluated by a collaborative study involving a total of 15 analysts in the USA and other countries. The method

is described in '*Compendium of methods for the microbiological examination of foods*'(1976) pp. 387–416 edited by M. L. Speck, American Public Health Association, Washington, DC. 4 foods (crabmeat, milk, ground beef, potato), either with or without enterotoxins, A, B, C, D and E added, were used in the collaborative study. Evaluation of the method was based on: solubility of food eluates after extraction and separation of enterotoxins; efficiency in recovering enterotoxins from foods; and serological categorizing of foods, as determined by the presence or absence of enterotoxins in the foods. Overall positive rates found for these 3 criteria were, resp. 94.79, 72.69 and 99.23%. It is concluded that the method is effective in separating staphylococcal enterotoxins from foods. The AOAC has adopted the method as official first action and no adverse comments have been received. The official first action method is detailed under the headings: apparatus; reagents; extraction of toxin; purification of toxin; and concn. of toxin. [See also FSTA (1976) 8 11C531 and *Journal of the Association of Official Analytical Chemists* (1980) 63, 413–415.] JA

24

[Staphylococcal food poisoning.] Staphylokokken als Lebensmittelvergifter. [Review]
Sinell, H.-J.
Ernährung 5 (4) 180–184 (1981) [17 ref. De] [Inst. für Lebensmittelhygiene der Freien Univ., Berlin]

Aspects relating to food poisoning caused by staphylococci are reviewed, covering: characteristics of the enterotoxins; symptoms of enterotoxin poisoning; mode of action of the toxin; factors affecting formation of staphylococcal enterotoxins in foods (contamination source, environmental preconditions, enterotoxin heat resistance, and rate of occurrence); detection methods; and preventative measures. RAW

25

[Outbreaks of food poisoning attributed to *Bacillus cereus* in Osaka city.]
Yasukawa, A.
Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi] 20 (3) 186–191 (1979) [18 ref. Ja, en] [Osaka City Inst. Public Health & Environmental Sci., Tojo-cho, Tennoji-ku, Osaka, Japan]

During 2 outbreaks of food poisoning due to *B. cereus*, 9 out of 13 employees in 2 factories became ill 0.5–3 h after taking lunch, and 211 out of 1809 persons who had eaten packed lunches complained of nausea and vomiting after 5–6 h. In both cases, *B. cereus* was isolated from the suspected lunches and from faeces of the patients (bacterial counts 1.3×10^4-4.7×10^8/g). In 2 cases culture supernatants of the isolates showed heat-sensitive lethal activity against mice on intraperitoneal or intravenous injection. TM

26

Method for inhibiting the growth of *Clostridium botulinum* and the formation of enterotoxin in smoked meat products.
Jadlocki, J. F., Jr.; Thompson, J. S. (FMC Corp.)
United States Patent 4 282 260 (1981) [En]

The growth of *Clostridium botulinum* and the production of enterotoxin in smoked meat products, such as bacon, is inhibited by adding to the meat products a compound, i. e. hypophosphorous acid or its Na, K, Ca or Mn salts. AS

27

Review of the potential hazard from botulism in cured meats. [Review]
Holley, R. A.
Canadian Institute of Food Science and Technology Journal 14 (3) 183–195 (1981) [122 ref. En, fr] [Food Res. Inst., Agric. Canada, Ottawa, Ontario K1A 0C6, Canada]

The degree of hazard presented by *Clostridium botulinum as* a result of cured meat production at decreased or non-inhibitory concn. of nitrite is strongly influenced by the number of contaminating spores present, pH, salt concn. and water activity of the meat as well as the length and temp. of storage. Availabe information indicates that *C. botulinum* spores and vegetative cells occur in raw and processed meats with low frequency (0.04–2.18/kg.) Nitrite appears to inhibit *C. botulinum* spore outgrowth by interfering with some essential Fe-containing compound such as ferredoxin, which impairs energy yielding reactions. Synergistic reactions of nitrite with ascorbate, EDTA, sorbate and acidification are discussed as well as blocking reactions of nitrite action by Fe and reversal of this reaction by polyphosphate. While it appears that the use of sorbic acid as an adjunct to nitrite causes inhibition of *C. botulinum* in cooked bacon produced in N. America, there appears to be little enthusiasm for its use in uncooked bacon or pasteurized cured meats in Europe, due in part to uncertainly regarding the toxicity of its reaction products and poor performance against clostridia. Biological acidification of bacon by starter cultures is a promising alternative for the production of safe, low-nitrite bacon. Nisin may prove to be of some value in decreasing the heat process or nitrite concn. required for production of semi-preserved meats. The need for use of lowered nitrite concn. because of nitrosamine formation is discussed for major product categories. Specific problem areas are identified and some suggestions for future research are made. AS

28

Growth effect of sorbate and selected antioxidants on toxigenic strains of *Staphylococcus aureus*.
Lahellec, C.; Fung, D. Y. C.; Cunningham, F. E.
Journal of Food Protection 44 (7) 531–534, 538 (1981) [14 ref. En] [Dep. of Anim. Sci. & Ind., Kansas State Univ., Manhattan, Kansas 66506, USA]

Effect of potassium sorbate with and without added antioxidants on *S. aureus* 196, S-6, 137 and 326 in a liquid system was evaluated. Potassium sorbate at 1, 3,

and 5% levels in combination with BHA, BHT, propyl gallate (PG) (50 and 100 p.p.m.) exerted greater bactericidal and bacteriostatic effects on *S. aureus* strains at pH 5 than at pH 7; at pH 6 the effect was more pronounced at 3 and 5% compared with 1% sorbate. TBHQ was highly inhibitory to *S. aureus* strains with or without the addition of sorbate. In combination with sorbate, BHA exerted greater bactericidal effects compared with BHT and PG. Higher concn. of antioxidants exerted more bactericidal and bacteriostatic effects on test organisms. *S. aureus* S-6 was more resistant than 196, 326, and 137 in the presence of sorbate and antioxidants. Shake cultures of *S. aureus* grew better than static cultures in the presence of sorbate and BHA. AS

29

Heat resistance of proteolytic *Clostridium botulinum* type F in phosphate buffer and crabmeat.
Lynt, R. K.; Kautter, D. A.; Solomon, H. M.
Journal of Food Science 47 (1) 204–206, 230 (1982) [En] [Div. of Microbiol., FDA, Washington, DC 20204, USA]

Heat resistance of proteolytic *C. botulinum* type F strains Langeland, PC and 4YRC was studied by the thermal death time tube method in phosphate buffer; 4YRC was also studied in crabmeat. Correction of exposure time for thermal lag and lethality during lag was made by the graphic method. Decimal reduction times in min (D) were calculated by Schmidt's probability method, using the Karber LD_{50}. For these 3 strains, D curves in phosphate buffer traversed one log cycle in 18.1–25.3°F (z); extrapolated they gave 0.14–0.23 for D_{250}. The z of the 4YRC crabmeat D curve was 23, extrapolating to 0.17–0.18 for D_{250}. Proteolytic type F therefore, resembled other proteolytic *C. botulinum* types. IFT

30

[Botulism. Current situation in relation to food poisoning by *Clostridium botulinum*. New results concerning toxigenesis. Detection of spores of *Clostridium botulinum*, and detection of toxin.]
Billon, J.
RTVA 20 (172) 5–8, 10–13 (1981) [35 ref. Fr] [Min. Agric., Direction de la Qualite, Lab. Cent. d'Hygiene Alimentaire, 43 Rue de Dantzig, 75015 Paris, France]

Aspects considered include: epidemiological aspects of botulism; characteristics of *Clostridium botulinum;* mode of action of the toxin; conditions for growth of, and formation of toxin by, *Cl. botulinum* (with reference to effects of temp., pH, NaCl, and other inhibitory factors); changes in toxicity with time; foods commonly responsible for outbreaks of botulism; honey in relation to botulism of infants; detection and counting of spores and vegetative cells of *Cl. botulinum;* and detection and typing of *Cl. botulinum* toxin in foods and cultures. AJDW

31

Immunodiffusion method for detection of type A *Clostridium botulinum*.
Ferreira, J. L.; Hamdy, M. K.; Zapatka, F. A.; Hebert, W. O.
Applied and Environmental Microbiology 42 (6) 1057–1061 (1981) [18 ref. En] [US FDA, Atlanta, Georgia 30309, USA]

A simple gel immunodiffusion agar procedure was developed for detecting toxigenic strains of *C. botulinum* type A (CbA). The method consisted of overlaying colonies grown on thin-layer tryptone-peptone-glucose-yeast extract agar with gel diffusion agar containing desired levels of CbA antitoxin. Concentric precipitin zones formed around colonies of CbA. Strains of CbA were detected by this procedure. However, *C. botulinum* type B reacted to a lesser degree with this system. No reaction was noted with types E, F Langeland, F_{8G}, *C. perfringens,* or with strains of nontoxigenic *C. sporogenes*. Thickness of the plating medium, incubation time and temp., environmental growth conditions, and levels of both agar and antitoxin were important factors affecting the efficiency of the procedure, whereas the age of the culture (used as inoculum) was not critical. Thin agar medium (5 ml per plate [15 by 100 mm]) containing 1.5% agar gave consistent results, but more agar limited diffusion, and lower levels encouraged spreaders. The optimal concn. of antitoxin incorporated into the gel diffusion agar overlay was 1.2 IU/ml gel diffusion agar. Rabbit type A antitoxin prepared with purer immunizing agent gave similar reactions. The addition of type A antitoxin in tryptone-peptode-glucose-yeast extract agar medium before inoculation with CbA showed promising results. AS

32

Detection of *Clostridium botulinum* type G toxin by enzyme-linked immunosorbent assay.
Lewis, G. E., Jr.; Kulinski, S. S.; Reichard, D. W.; Metzger, J. F.
Applied and Environmental Microbiology 42 (6) 1018–1022 (1981) [10 ref. En] [Dep. of Applied Toxin Res., US Army Med. Res. Inst. of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701, USA]

The enzyme-linked immunosorbent assay (ELISA) procedure described provides a rapid, sensitive diagnostic test for identification of *C. botulinum* toxin type G (CbG) in foods and confirmatory diagnosis of intoxication in humans. With the double-sandwich technique and alkaline phosphatase as the enzyme indicator, CbG was detected in quantities equaling those required for one mouse intraperitoneal median lethal dose. The procedure took approx. 6.5 h, but this could be reduced to ≤ 5.5 h with the use of precoated plates stored at −70°C. Cross-reactions did not occur with culture extracts of *C. sporogenes* or *C. botulinum* types B, C, D, E, and F. Acidic preparations of *C. botulinum* type A exhibited nonspecific reactivity. Likewise, 50% of the *C. subterminale* isolates tested were cross-reactive in the assay. These latter isolates express similar metabolic and physiological characteristics with CbG. AL

33

Growth and toxin formation by *Clostridium botulinum* at low pH values.
Smelt, J. P. P. M.; Raatjes, G. J. M.; Crowther, J. S.; Verrips, C. T.
Journal of Applied Bacteriology **52** (1) 75–82 (1982) [23 ref. En] [Unilever Res. Lab., Vlaardingen, Netherlands]

Spores of *Clostridium botulinum* were found to initiate growth and to produce toxin in aqueous suspensions of soy protein at pH values as low as 4.2 and in skim milk at pH 4.4. Most of the experiments were done with mixed cultures of *Cl. botulinum* types A and B in the presence of 2 strains of *Bacillus subtilis*. The role of the latter organism was concluded to be to lower the oxygen content and the E_h of the suspensions. Toxin was produced at pH 4.4 after 4 wk of incubation at 30°C when either HCl or citric acid were used as the acidulant and after 12 and 14 wk when resp., lactic and acetic acids were used. Thus, amongst other factors the nature of the acid and not solely the pH value is an important factor in controlling the growth of *Cl. botulinum* at low pH. Pure cultures of *Cl. botulinum* type A grew at 30°C under strictly anaerobic conditions and produced toxin at pH 4.3 in the presence of HCl. AS

34

[A simple method for detection of staphylococcal enterotoxin B in vanilla custard by the ELISA test.] Einfache Methode für den Nachweis von Staphylokokken-Enterotoxin-B in Vanillepudding mittels ELISA-Test.
Büning-Pfaue, H.; Timmermanns, P.; Notermans, S.
Zeitschrift für Lebensmittel-Untersuchung und Forschung **173** (5) 351–355 (1981) [17 ref. De, en] [Zoonoses & Food Microbiol. Lab., Nat. Inst. of Public Health, Bilthoven, Netherlands]

The ELISA (enzyme-linked immunosorbent assay) test [FSTA (1981) 13 1C7] was used to detect staphylococcal enterotoxin B (SEB) in Dutch vanilla custard (vla). This product, made from milk plus starch, egg yolk, sugar and vanilla, was chosen because it had been the source of an outbreak of food poisoning due to *Staphylococcus aureus* [FSTA (1981) 13 11C370]. Two 100-g samples, containing resp. 5 µg added SEB and 6–7 µg SEB formed by *S. aureus* cultures, were prepared by a procedure involving extraction at pH 7.4 and then at pH 4.5, dialysis overnight in flowing water, further extraction at pH 4.5, neutralization, filtration, and concentration of the filtrate in 35% polyethylene glycol 20 000 solution. Another 3 samples containing 6–7 µg culturally formed SEB were prepared by shortened versions of this procedure. Good results were obtained by a shortened procedure involving extraction at pH 7.4 and 4.5, followed by neutralization and concentration (1:20). In 2 samples containing only 0.1 and 0.5 µg added SEB/100 g, this procedure detected 0.04 and 0.25 µg resp. With a detection limit of about 3 ng/ml, the ELISA test was about 200 times more sensitive than the Ouchterlony test. ADL

35

[Death of one child and severe poisoning of another after consumption of canned black pudding (Blutwurst) containing botulinus toxin formed by faulty manufacture and storage techniques.] Bildung von Botulinus-Toxin durch fehlerhafte Herstellung und Lagerung von Dosenblutwurst, deren Verzehr bei einem Kind zum Tode und bei einem anderen Kind zu schweren Vergiftungserscheinungen führten.
Coduro, E.
Zeitschrift für das Gesamte Lebensmittelrecht (ZLR) **8** (2) 165–173 (1981) [De]

36

[Staphylococcal intoxication caused by cooked Kasseler.] Gegartes Kasseler als Ursache einer Staphylokokkenintoxikation.
Untermann, F.; Bornemann, H.; Kusch, D.
Fleischwirtschaft **61** (11) 1652–1653; 1712 (1981) [9 ref. De, en] [Landesanstalt für Veterinärmedizin & Lebensmittelhygiene, Invalidenstrasse 60, D-1000 Berlin 21]

4 cases of staphylococcal food intoxication attributed to consumption of grilled Kasseler (cured pork) are used to describe the health hazard due to keeping meat products in heated display cabinets, and to emphasize the importance of regular testing. Instant action thermometers are recommended for temp. control of the stored meat products. Bacteriological examination showed that the intoxication was due to 2 enterotoxin A-forming strains of staphylococci which differed in their phage types and lecithinase activity. The case shows that bacteriological analysis of meat products, using egg yolk selective media, should include tests for lecithinase-negative colonies. Temp. detn. on products in the heated cabinets showed temp. of 41° to 65°C, Kasseler temp. were mainly 45°–50°C, which allows bacterial growth. RM

37

Hazard analyses, in reference to *Bacillus cereus*, of boiled and fried rice in Cantonese-style restaurants.
Bryan, F. L.; Bartleson, C. A.; Christopherson, N.
Journal of Food Protection **44** (7) 500–512 (1981) [26 ref. En] [US Dep. of Health & Human Services, Centers for Disease Control, Atlanta, Georgia 30333, USA]

Hazard analyses were conducted in six Cantonese-style restaurants to evaluate the amount of *B. cereus* in rice and the water activity and temp. of rice at each stage of processing. Each of 16 samples of raw, polished rice contained *B. cereus*. The water activity of cooked rice ranged from 0.912 to 0.980, and was related to the stage of the processing and storage practice. Rice reached temp. > 93°C (200°F) during cooking. Cooked rice held in steam tables was maintained at temp. that should preclude growth of *B. cereus*. Whenever cooked rice was kept at room temp. for a few hours, the temp. became such that considerable growth of *B. cereus* could have occurred. Rice in layers < 9 cm thick cooled rather rapidly; layers thicker than 9 cm cooled more

slowly. During frying and refrying, temp. exceeded 74°C (165°F) *B. cereus* was frequently isolated from rice at various stages of preparation and storage, but in numbers $<10^3$/g. This organism was also isolated from rice storage pans. Recommendations for preventing problems that could be caused by *B. cereus* as a result of preparation and storage practices are to cook only small batches of rice at intervals during the day; hold cooked rice at or above 55°C (131°F); cool cooked rice in shallow pans in layers <9-cm thick; and fry rice so that every grain is certain to reach a temp. of at least 74°C (165°F). AS

38

Variation in counts, enterotoxin levels and TNase in Swiss-type cheese contaminated with *Staphylococcus aureus*.

Todd, E.; Szabo, R.; Robern, H.; Gleeson, T.; Park, C.; Clark, D. S.

Journal of Food Protection 44 (11) 839–846, 851–852, 856 (1981) [42 ref. En] [Bureau of Microbial. Hazards, Health & Welfare Canada, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada]

In Swiss-type cheeses, produced by 1 manufacturer between Jan. and April 1977 and withdrawn from sale because of contamination with *Staphylococcus aureus*, counts of *S. aureus* ranged from 25 to 10^8/g in the 59 cheese lots examined; most samples had counts of 10^4–10^6/g. Enterotoxin B was identified in 73% of 186 samples and thermonuclease (TNase) was found in 53% of 122 samples. Variations in *S. aureus* counts, enterotoxin and TNase contents and in pH were observed within lots and between the centre and periphery of individual cheeses. Reasons for these variations are discussed. TNase was not detectable in samples with an enterotoxin B content of <1 μg/g. This amount of enterotoxin can be a health hazard. Some recommendations for examination of suspect cheeses are made, and acceptable limits of contamination are suggested. Contamination of the starter culture with *S. aureus* was the probable cause of the cheese contamination. MEG

39

A food poisoning whodunit.

Corwin, E.

FDA Consumer 14 (9) 4–7 (1980) [En]

In July 1978 a can of salmon contaminated with botulinal toxin caused the death of 2 people in Birmingham, UK. Details of the exhaustive international investigations into how the contamination occurred are presented. VJG

40

Heat inactivation and reactivation of alpha toxin from *Clostridium perfringens*.

Babajimopoulos, M.; Mikolajcik, E. M.

Journal of Food Protection 44 (12) 899–902 (1981) [15 ref. En] [Dep. of Food Sci. & Nutr., Ohio Agric. Res. & Development Cent., Columbus, Ohio 43210, USA]

Because of its potential use as an indicator of *C. perfringens* presence or growth in foods, the heat stability of alpha toxin from *C. perfringens* was studied at 55–95°C in the presence or absence of proteose peptone fractions. D values for thermal inactivation under such conditions are tabulated. Presence of proteose peptone fractions with mol. wt. >50 000 protected alpha toxin against inactivation at ≤85°C; alpha toxin otherwise began to be inactivated at ≥65°C. Gel filtration showed that the protective effect was due to complex formation between protease peptone and alpha toxin. If alpha toxin was partially inactivated by heating for various times at 55–95°C, partial recovery of activity was obtained by addition of proteose peptone followed by incubation on haemolysin indicator (H1) plates at 37°C for 48 h. This was solely due to the proteose peptone and not the incubation. It is concluded that the toxin could retain activity in some heat-treated foods, and could be used in the H1 test for growth of *C. perfringens*. DIH

41

Effect of glyceryl monolaurate on toxin production by *Clostridium botulinum* in meat slurry.

Notermans, S.; Dufrenne, J.

Journal of Food Safety 3 (2) 83–88 (1981) [13 ref. En] [Nat. Inst. of Public Health, PO Box 1, 3720 BA Bilthoven, Netherlands]

Glyceryl monolaurate when used in the proportion of 5 g/kg meat slurry (pH 6.0–6.2) inhibited toxin production by *C. botulinum* type A (strain 73A), type B (strain OKRA) and type E (strain RIV 2). The concn. of glyceryl monolaurate required for inhibition was not reduced by addition of lactic acid to the meat slurry until the pH was lowered to ≤ pH 5.2. In meat slurry (pH 6.0–6.2), potassium sorbate inhibited production of type B toxin at 32 g/kg. The addition of butylated hydroxyanisole to glyceryl monolaurate had no effect upon the concn. needed for inhibition of botulinum toxin production. AS

42

Enterotoxin production by *Staphylococcus aureus* related to the origin of the strains.

Olsvik, O.; Berdal, B. P.; Fossum, K.; Omland, T.

Acta Pathologica et Microbiologica Scandinavica, B 89 (6) 423–426 (1981) [23 ref. En] [Norwegian Defence Microbiol. Lab., Nat. Inst. of Public Health, Geitmyrsveien 75, Oslo 4, Norway]

35 strains of *Staphylococcus aureus* of human origin, 24 strains isolated from food, either in connection with outbreaks of human intoxication or by routine control, and 111 strains from various animal spp. were examined for enterotoxin production. Using an enzyme-linked immunosorbent assay technique for detection, 104 of the 170 strains produced ≥ 1 staphylococcal enterotoxin. Enterotoxin C prevailed in strains from cows and milk, while enterotoxin A was associated with human food poisoning. AS

43

Production of botulinum toxin in inoculated pack studies of foil-wrapped baked potatoes.
Sugiyama, H.; Woodburn, M.; Yang, K. H.; Movroydis, C.
Journal of Food Protection 44 (12) 896–898, 902 (1981) [11 ref. En] [Food Res. Inst., Univ. of Wisconsin, 1925 Willow Drive, Madison, Wisconsin 53706, USA]

Idaho Russet Burbank potatoes were surface or stab inoculated with 10 to 10^5 spores of *Clostridium botulinum* type A strain, overwrapped in Al foil, baked at 204°C for 50 min or 96°C for 3 h and then held at 22° or 30°C. The shortest incubations resulting in the first botulinogenic potatoes were inversely related to spore doses and ranged from 3 to 7 days; potatoes inoculated with 10 spores were toxic after 5 to 7 days. Total toxin in individual potatoes incubated 3 to 5 days were 5×10^3 to 5×10^5 mouse mean lethal doses. Toxin was not found at distances greater than 1.6 cm from the spore inoculation site. Results indicate that left-over, foil-wrapped, baked potatoes are a perishable food that must be refrigerated. AS

44

Public health significance of milk borne intoxications. II. *E. coli, B. cereus, Streptococcus* and *Aspergillus*.
Batish, V. K.; Garg, S. K.; Harish Chander; Ranganathan, B.
Indian Dairyman 33 (8) 501–503 (1981) [4 ref. En] [Dairy Bact. Div., Nat. Dairy Res. Inst., Karnal-132 001, India]

Nature and biological activity of toxins produced by *Escherichia coli, Bacillus cereus, Streptococcus* and *Aspergillus* spp. in milk and milk products are reviewed, and preventive measures are outlined. CFTRI

45

Production of Italian dry salami: effect of starter culture and chemical acidulation on staphylococcal growth in salami under commercial manufacturing conditions.
Metaxopoulos, J.; Genigeorgis, C.; Fanelli, M. J.; Franti, C.; Cosma, E.
Applied and Environmental Microbiology 42 (5) 863–871 (1981) [24 ref. En] [Dep. of Epidemiology & Preventive Med., Univ. of California, Davis, California 95616, USA]

The effect of starter culture and chemical acidulation on the growth and enterotoxigenesis of *Staphylococcus aureus* strain S-6 in Italian dry salami under commercial manufacturing conditions was studied. The experimental design included 2 levels of *S. aureus* (10^4 and 10^5/g), 3 levels of starter culture (0, 10^5, and 10^6/g), 3 levels of initial pH (pH_0) 6.1, 5.5, and 4.8), 2 manufacturing plants, and 3 replications. *S. aureus* growth in the salami was affected significantly ($P < 0.005$) by pH_0, initial levels of *S. aureus* ($staph_0$) and lactic acid bacteria (LAB_0), day of fermentation, and by the interactions of $pH_0 \times$ day, $pH_0 \times LAB_0$, $LAB_0 \times staph_0$, $pH_0 \times staph_0$, and $pH_0 \times$ location of fermentation. In general, the lower the pH_0 and the higher the LAB_0, the greater the inhibition of *S. aureus*. The LAB levels during the fermentation were affected significantly ($P < 0.005$) by pH_0, LAB_0, day of fermentation, location, $LAB_0 \times pH_0$, and $LAB_0 \times$ day. Derived regression equations related level of *S. aureus* and LAB at any day of fermentation to a number of microbiological and chemical variables. Close similarity of observed and predicted levels of *S. aureus* and LAB growth demonstrated the usefulness of the experimental approach in evaluating the safety of a process. No detectable enterotoxin or thermonuclease was found at any stage of processing even when *S. aureus* reached levels of 10^7/g of salami. AS

46

The microbiological safety of canned, cured, perishable meat products. [Lecture]
Johnston, R. W.; Krumm, G. W.
Proceedings of the European Meeting of Meat Research Workers No. 26, Vol. II, N-12, pp. 295–299 (1980) [13 ref. En] [USDA, Food Safety & Quality Service, Washington, DC, USA]

Potential health hazard from pasteurized canned hams and similar products, exposed to temp. abuse during storage and distribution, is discussed with special reference to *Clostridium botulinum*. Data for brine concn. in imported and US domestic hams over the period 1973–1977 are presented. Average brine % was slightly higher in imported than domestic hams. For both, large numbers had brine % lower than the desirable level of 3.5%, and some were below 1.2%. As nitrite concn. is generally closely related to brine concn., some canned hams would have little or no anticlostridial activity, presenting a health hazard if not refrigerated throughout distribution. Various cases of spoilage of pasteurized canned hams in the USA are discussed. It is concluded that the preservative effect of salt and nitrite may not be as effective as commonly believed, because of variation in concn. of these additives in the product, and loss of nitrite during storage. [See FSTA (1982) 14 8S1379.] AJDW

47

Effect of sulfur dioxide on *C. botulinum* in cooked meat. [Lecture]
Tompkin, R. B.; Christiansen, L. N.; Shaparis, A. B.
Proceedings of the European Meeting of Meat Research Workers No. 26, Vol. II, M-11, pp. 248–249 (1980) [6 ref. En] [Swift & Co., 1919 Swift Drive, Oak Brook, Illinois 60521, USA]

A canned comminuted pork product was prepared with 50, 100 or 200 μg SO_2/g, or 200 μg SO_2 + 40 μg $NaNO_2$/g, inoculated with a heat shocked culture of 5 *Clostridium botulinum* type A and 5 type B strains, canned, stored at 4.4°C for 0, 10 or 26 wk, and incubated at 27°C. Swelled cans were tested for *Cl. botulinum*

toxin. 200 μg SO_2/g gave considerable inhibition of *Cl. botulinum* toxin formation; lower SO_2 concn. were much less effective. Addition of 40 μg $NaNO_2$/g considerably reduced the inhibitory activity of 200 μg SO_2/g. Holding at 4.4°C also reduced the inhibitory activity of SO_2, although considerable inhibitory activity remained after 26 wk. Variability of inhibitory activity is also discussed. [See FSTA (1982) 14 8S1379.] AJDW

48

[Evaluation of coagulase tests for pathogenic staphylococci.]
Pinto, M. A. O.; Ferreiro, L.; Santos, E. C. dos
Arquivos da Escola de Veterinaria da Universidade Federal de Minas Gerais 33 (1) 207–212 (1981) [17 ref. Pt, en] [Pesquisadora da EPAMIG/DTA/ILCT, Juiz de Fora, MG, Brazil]

3 different methods for detecting coagulase (the tube test, slide test and plasma agar test) were compared, using 488 strains of *Staphylococcus aureus* isolated from mastitic milk, raw milk and Minas cheese. There was a 92.4% agreement between the plasma agar test for identification of pathogenic staphylococci (Boothby et al., FSTA (1979) 11 9B91) and the tube test, 89.5% agreement between the plasma agar and the slide tests and 86.7% agreement between the tube and slide tests. The plasma agar test is recommended for detection of pathogenic *S. aureus* strains. MEG

49

[Growth and toxin production of *Clostridium botulinum* in food.]
Yokoseki, M.; Fujii, T.
Journal of Japanese Society of Food Science and Technology [Nippon Shokuhin Kogyo Gakkaishi] 24 (8) 420–431 (1977) [89 ref. Ja] [Tokai Regional Fisheries Res. Lab., Kachidoki-5, Chuo, -ku, Tokyo, Japan]

50

[Survey on contamination of commercial foods with *Bacillus cereus*, and biotypes and serotypes of the isolates.]
Ushioda, H.; Igarashi, H.; Takahashi, M.; Shingaki, M.; Terayama, T.; Sakai, S.
Annual Report of Tokyo Metropolitan Research Laboratory of Public Health 31 (1) 109–114 (1980) [17 ref. Ja] [Metropolitan Res. Lab. of Public Health, Hyakunincho 3 chome, Shinjuku-ku, Tokyo, Japan]

Between Jan. 1977 and Dec. 1979 *Bacillus cereus* was found in the following number of samples of foods sold in Tokyo: tofu 887 of 1217; raw vegetables 84 of 203; powdered baby food 41 of 164; snack foods 19 of 119; western type confections 153 of 1016; noodles 97 of 697; packed lunches 87 of 636; bread 114 of 1046; salads 85 of 855; Japanese type confections 39 of 456; cooked vegetables 86 of 998; spaghetti 4 of 75; cooked bean products 1 of 196. There were wide variations among *B. cereus* positive samples in their counts of *B. cereus* (10^2–10^6/g) and coliforms (0–10^7/g), but except for tofu and confections, positive samples tended to have higher total bacterial counts and counts of coliforms than negative samples. Of 155 strains of *B. cereus* 63 were classified into 6 groups according to saccharose, cellobiose and salicin fermentation, starch, escalin and urea hydrolysis, and lecithinase reaction: 96 strains were classified according to their haemolytic pattern, with the largest number of strains (29) belonging to Taylor & Gilbert's group. CIH

51

[Effect of sucrose on growth of *Staphylococcus aureus* and enterotoxin production in egg custard.]
Igarashi, H.; Shingaki, M.; Takahashi, M.; Ushioda, H.; Terayama, T.; Sakai, S.
Annual Report of Tokyo Metropolitan Research Laboratory of Public Health 31 (1) 122–126 (1980) [Ja, en] [Tokyo Metropolitan Res. Lab. of Public Health 24-1, Hyakunincho 3 chome, Shinjuku-ku, Tokyo 160, Japan]

When sucrose was added to egg custard at a concn. of 30%, growth of *Staphylococcus aureus* strains 243 and FRI-361 (producing enterotoxins B and C resp.) was partially inhibited at 25°C and production of enterotoxin B during incubation at 25°C for 48 h was reduced. [From En summ.] MEG

52

Factors affecting growth and toxin production by *Clostridium botulinum* type E on irradiated (0.3 Mrad) chicken skins.
Firstenberg-Eden, R.; Rowley, D. B.; Shattuck, E. G.
Journal of Food Science 47 (3) 867–870 (1982) [En] [US Army Natick R&D Lab., Food Microbiol. Group, Sci. & Advanced Tech. Lab., Natick, Massachusetts 01760, USA]

A model system (chicken skins with chicken exudate) was used to determine if *Clostridium botulinum* type E (Beluga) spores, stressed by low dose irradiation, would develop and produce toxin at abuse temp. of 10° and 30°C in the absence of characteristic spoilage. Unstressed spores germinated, multiplied, and produced toxin on vacuum-packaged chicken skins, stored at either 30° or 10°C. Cell numbers increased faster and toxin was evident sooner at 30° than at 10°C. At 30°C, growth occurred and toxin was produced more slowly when samples were incubated aerobically than anaerobically. When samples were incubated aerobically at 10°C, no toxin was detected within a test period of 14 days. An irradiation dose of 0.3 Mrad at 5°C reduced a spore population on vacuum-sealed chicken skins by about 90%. The surviving population produced toxin at 30°C under either aerobic or anaerobic conditions; at 10°C no toxin was detected even on skins incubated anaerobically. Under the worst conditions (30°C, vacuum packaged) toxin was not detected prior to characteristic spoilage caused by the natural flora surviving 0.3 Mrad. IFT

53

Thermal inactivation of *Clostridium perfringens* enterotoxin in buffer and in chicken gravy.
Bradshaw, J. G.; Stelma, G. N.; Jones, V. I.; Peeler, J. T.; Wimsatt, J. C.; Corwin, J. J.; Twedt, R. M.

Journal of Food Science 47 (3) 914-916 (1982) [En] [Food & Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226, USA]

Time-temp. relationships for inactivation of purified enterotoxin prepared from *C. perfringens* were determined by Vero cell assay in 3 different heating menstrua. Enterotoxin diluted to an initial concn. of 12.5 µg/g in chicken gravy and in 0.1M phosphate buffer, both at pH 6.1, was heated at temp. of 59-65°C. Average inactivation times in gravy ranged from 1.5 to 72.8 min and in buffer from 2.4 to 149.4 min. IFT

54

Effects of *Clostridium botulinum* toxins on infant and adult mice.
Wisdom, C.; Midura, T. F.
Applied and Environmental Microbiology 43 (4) 955-957 (1982) [8 ref. En] [California Dep. of Health Services, Berkeley, California 94704, USA]

The *C. botulinum* cultures tested contained isolates from foods, wounds, faeces of infants with infant botulism, etc. Toxic supernatants of many *C. botulinum* type B isolates from various sources appeared to be more toxic in infant (I) mice than adult (A) mice. When type B isolates from 17 food-associated botulism cases were tested, the culture supernatant fluids from 7 isolates (41%) had I/A LD_{50} ratios of ≤ 8, but 10 isolates (59%) appeared to be at least 15× more lethal in I than A, and one isolate (isolate 185, from canned ham) had an I/A LD_{50} ratio of 280. The significance of the finding that infant mice were more sensitive indicators of toxicity than adult mice in diagnostic situations is discussed. AL

55

Growth and enterotoxin production of *Staphylococcus aureus* (isolated from heat treated milk in a ten-month survey) in the manufacture of Cheddar cheese made from heat treated milk.
Lam, N. C. S. I.
Dissertation Abstracts International, B 42 (2) 551 (1981) [En] [Univ. of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada]

A heat-resistant enterotoxin A-producting *Staphylococcus aureus* produced detectable amounts of enterotoxin in raw milk 6-8 h after inoculation of 10^3-10^5 *S. aureus*/ml, when the *S. aureus* count had reached 13 million/ml raw milk. In heat-treated milk (61°C for 18 s), enterotoxin was detected after 4-6 h, when *S. aureus* count had reached 2.8 million/ml. Indigenous *S. aureus* present in raw milk produced enterotoxin 12 h after heat treatment of the milk (at 61°C for 18 s), when *S. aureus* count reached 82.5 million/ml. Enterotoxin was detected earlier in cheeses made with a slow starter. As few as 3.4 million *S. aureus*/g cheese resulted in enterotoxin production in slow cheese vs. > 29 million/g in cheese made with normal starter. In cheese with partial starter failure, enterotoxin was detected when *S. aureus* count reached 12 million/g. Cheeses containing *S. aureus* had normal moisture and salt content, but at any *S. aureus* inoculum level, those made with normal starter had a higher fat content than those made with slow starter. The feasibility of solid-phase radioimmunoassay for qualitative and quantitative detection of enterotoxin A in cheese was demonstrated. CDP

56

[Botulism after consumption of raw hams. Experimental study.] Botulismus nach Verzehr von Rohschinken. Experimentelle Untersuchungen.
Lücke, F.-K.; Hechelmann, H.; Leistner, L.
Fleischwirtschaft 62 (2) 203-306 (1982) [many ref. De, en] [Inst. für Mikrobiologie, Toxikologie und Histologie, Bundesanstalt für Fleischforschung, 8650 Kulmbach, Federal Republic of Germany]

In curing experiments with hams inoculated with *Clostridium botulinum* spores, it was observed that non-proteolytic strains (types B and E) grew and formed toxin in the interior of large bone-in hams if the curing temp. was 8° or 10°C. A curing temp. of 5°C only provided safety if it was maintained for a sufficiently long time: a NaCl content of 2.8% did not prevent growth of the non-proteolytic *C. botulinum* strains during subsequent smoking and storage at 20°C. Toxin formation was not significantly decreased or delayed when different curing procedures (dry-curing or brine-curing) or different amounts of nitrite and nitrate in the curing salt were employed, or when Enterobacteriaceae were also present at the inoculated sites. After manufacture, the toxic hams had only a slight off-odour and were not putrid; however, they contained between 600 and 35 000 times the dose of mouse-LD_{50} of toxin at the inoculation sites (determined after trypsin treatment of the extracts). Results agree with diagnostic observations reported in the literature and support the belief that psychrotrophic strains of *C. botulinum* type B are responsible for many outbreaks of botulism due to the consumption of raw hams. AS

57

A simple medium for isolation of coagulase-positive staphylococci in a single step.
Mintzer-Morgenstern, L.; Katzenelson, E.
Journal of Food Protection 45 (3) 218-222 (1982) [10 ref. En] [A. Felix Public Health Lab., Min. of Health, PO Box 8255, Tel Aviv, Israel]

An agar medium containing NaCl, egg yolk and tellurite for selective quantitative isolation of coagulase-positive staphylococci from food was developed. Isolation and identification of the staphylococci was achieved in a single step. A properly diluted food sample was spread over the medium and incubated for 24 h at 42°C. Coagulase-positive, staphylococci appeared as small grey to dark-grey colonies surrounded by a dense white opacity. Coagulase-negative bacteria, which sometimes grow on this medium, did not produce this reaction. The identification, on this selective medium, of isolates from 683 different food samples as coagulase-positive

staphylococci was subsequently confirmed by the coagulase test. Comparative titrations of 29 coagulase-positive staphylococcal strains (isolated from meat, vegetables, cakes, cream, ice cream and human swabs) on both the selective medium and nutrient agar yielded nearly identical titres. Growth of heat-stressed staphylococci was inhibited by the selective medium. Complete reversal of the inhibition was achieved by a 3-h pre-incubation in brain heart infusion at 37°C. AS

58

Public health significance of *Staphylococcus hyicus* and intracellular staphylococcal enterotoxins in foods.

Adesiyun, A. A.

Dissertation Abstracts International, B 42 (6) 2317: Order no. 8125928, 116pp. (1981) [En] [Univ. of Minnesota, Minneapolis, Minnesota 55455, USA]

5 strains of *S. hyicus* (3 of subspecies *hyicus* and 2 of subspecies *chromogenes*) were examined for their ability to produce known and unknown enterotoxins. Of the 5 strains studied, 4 had been genetically characterized as being non-*S. aureus* species. None of the 5 strains produced detectable amounts of known enterotoxins but all 5 produced new or unknown enterotoxins. It is concluded that species other than *S. aureus* should be included in food safety surveillance programmes because of the potential hazard to humans of their enterotoxins. Studies of intracellular staphylococcal enterotoxin A (ISEA) released from *S. aureus* cells by lysostaphin lysis indicated that it is biologically active and could lead to food poisoning if sufficient numbers of *S. aureus* cells are consumed. *S. aureus* cells released ISEA in vitro within 30 min at 37°C when exposed to pancreatin at 6 mg/10^{10} cells. Release of ISEA in *S. aureus* cells was found to be affected by water activity, temp. and pH. JA

59

Enterotoxigenicity of *Staphylococcus aureus* strains isolated from poultry: raw poultry carcasses as a potential food-poisoning hazard.

Harvey, J.; Patterson, J. T.; Gibbs, P. A.

Journal of Applied Bacteriology 52 (2) 251–258 (1982) [34 ref. En] [Dep. of Agric. for N. Ireland, Newforge Lane, Belfast BT9 5PX, UK]

Staphylococcus aureus strains were isolated from end-of-lay poultry carcasses obtained from a plant at 2 different stages of processing before and after storage at different temp. These strains were supplemented with *S. aureus* strains isolated from poultry from a wide range of sources and biotyped, phage typed, and tested for production of enterotoxins A-E. The isolates were found to consist of poultry and human specific strains and each of these groups contained strains able to produce enterotoxin. Poultry strains produced only enterotoxin D whereas human strains produced enterotoxins A, C and D. The hen carcasses used in storage experiments were found to be naturally contaminated with enterotoxin D producing staphylococci. No enterotoxin D could be detected on any of the carcasses even after storage at temp. which allowed multiplication of the organisms to occur (final *S. aureus* counts ranged from 10^2 to 10^7/16 cm^2 of breast skin). AS

60

[Effect of X-ray irradiation of staphylococci on formation of enterotoxin and heat resistant deoxyribonuclease.]

Szulc, M.; Pliszka, A.; Peconek, J.

Medycyna Weterynaryjna 36 (9) 543–545 (1980) [13 ref. Pl, ru, en] [Katedra Higieny Produktow Zwierzecych, Wydzial Weterynaryjny, SGGW-AR, 02-528 Warsaw, Poland]

Staphylococcus aureus 262 (forming B enterotoxin) and *Staph. aureus* 100 (forming A enterotoxin) obtained from the collection of the Food Research Institute, University of Wisconsin, USA were exposed to X-ray irradiation at 100, 1000, 5000, 200 000 and 400 000 rad as described in the preceding abstr. in the 2 media stated there. 4 other *Staph. aureus* strains (3 collection and 1 isolated from meat) were used as controls. Extent of survival after irradiation was similar in all 6 strains; survival in broth was better than in saline. Irradiation at ≤400 000 rad did not inhibit enterotoxin formation by *Staph. aureus* bacteria multiplied from those of strains 262 and 100 that survived irradiation; irradiation in saline, but not in broth, inhibited production of heat stable deoxyribonuclease by the strains studied, the capacity being restored on transfer to broth culture. [See also FSTA (1981) 13 11B110–112.] SKK

61

[*Clostridium perfringens* toxins.] *Clostridium perfringens*-Toxine. [Review]

Katsaras, K.

Archiv für Lebensmittelhygiene 31 (4) 121–125 (1980) [many ref. De, en] [Bundesanstalt für Fleischforschung, 8650 Kulmbach, Federal Republic of Germany]

This review describes the 5 different types (A–E) of *Cl. perfringens* differentiated by type-specific antisera, and the disorders caused in man and animals. *Cl. perfringens* type A is one of the most common agents causing food poisoning in man, after consumption of ≥10^6 organisms/g of food. The production of enterotoxins in foods, detection of enterotoxin, and prevention of outbreaks of food poisoning by strict hygiene, heat treatment, pH and moisture control are briefly discussed. RM

62

Detection of staphylococcal enterotoxigenicity. III. Strains isolated in 1979–1980.

Petras, P.; Maskova, L.

Journal of Hygiene, Epidemiology, Microbiology and Immunology 26 (1) 95–101 (1982) [8 ref. En, fr, de, es] [Inst. of Hygiene & Epidemiology, Prague, Czechoslovakia]

Studies were conducted to test staphylococcus isolates for enterotoxigenicity, and to identify the toxins formed. 117 strains from food and 13 strains from swab samples from food processing premises were investigated over the period 1979–1980. None of the swab strains were enterotoxigenic. 34 of the food isolates were enterotoxigenic; enterotoxins A, B and D were resp. produced by 18, 5 and 11 isolates. None of the isolates formed enterotoxins C or E. Similar data are presented for the period 1975–1978. Data are also presented for enterotoxigenicity of isolates from veterinary and clinical sources. AJDW

63

The staphylococcal enterotoxins – a genetic overview.

Iandolo, J. J.; Dyer, D. W.

Journal of Food Safety 3 (4) 249–265 (1981, publ. 1982) [23 ref. En][Microbiol. Group, Kansas State Univ., Manhattan, Kansas 66506, USA]

64

DNA hybridization method for detecting enterotoxigenic *Escherichia coli* in human isolates and its possible application to food samples.

Hill, W. E.

Journal of Food Safety 3 (4) 233–247 (1981, publ. 1982) [51 ref. En][Div. of Microbiol., FDA, Washington, DC 20204, USA]

A DNA hybridization assay was used to detect isolates of *Escherichia coli* that have the genetic potential to produce toxin. This method was compared with the traditional Y-1 mouse adrenal cell method for detecting pathogenic *E. coli* isolated from humans. Results showed that the DNA hybridization method has the potential to detect foodborne bacterial pathogens. The advantages and disadvantages of this genetic method are compared with those of the traditional assays for pathogenicity. AS

65

[Honey and infant botul ism.]

Aureli, P.; Accorti, M.

Rivista della Societa Italiana di Scienza dell'Alimentazione 10 (3) 181–184 (1981) [It][Lab. Alimenti, Istituto Superiore di Sanita, Rome, Italy]

Aspects considered include: variability of honey; processing of honey; incidence of infant botulism; incidence of *Clostridium botulinum* in honey; spore germination and toxin formation in the gastrointestinal tract; sources of contamination of honey with *Cl. botulinum;* spore dose needed for infection; differences in susceptibility of infants to infection; and the protective effect of breast-feeding as compared to bottle-feeding. AJDW

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